Comprehensive analyses of transcriptomes induced by Lyme spirochete infection to CNS model system

Shiyuan Wen

Kunming Medical University

Xin Xu

Kunming Medical University

Jing Kong

Kunming Medical University

Lisha Luo

Kunming Medical University

Peng Yue

Kunming Medical University

Wenjing Cao

Kunming Medical University

Yu Zhang

Kunming Medical University

Meixiao Liu

Kunming Medical University

Yuxin Fan

Kunming Medical University

Jingjing Chen

Kunming Medical University

Mingbiao Ma

Kunming Medical University

Lvyao Tao

Kunming Medical University

Yun Peng

Kunming Medical University

Yan Dong

Kunming Medical University

Bingxue Li

Kunming Medical University

Suyi Luo

Kunming Medical University

Feng Wang

Kunming Medical University

Guozhong Zhou

Kunming Medical University

Taigui Chen

Kunming Medical University

Lianbao Li

Kunming Medical University

Aihua Liu

Kunming Medical University

Fukai Bao (✉ baofukai@kmmu.edu.cn)

Institute of Tropical Medicine <https://orcid.org/0000-0003-2652-6660>

Research Article

Keywords: Lyme neuroborreliosis, pathogenesis, transcriptomic analysis, *Borrelia burgdorferi*, candidate biomarker

Posted Date: September 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-822329/v1>

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Abstract

Background

Lyme disease is a zoonotic disease caused by infection with *Borrelia burgdorferi* (*Bb*), the involvement of the nervous system in Lyme disease is usually referred to as Lyme neuroborreliosis (LNB). LNB has diverse clinical manifestations, most commonly including meningitis, Bell's palsy, and encephalitis. However, the molecular pathogenesis of neuroborreliosis is still poorly understood. Comprehensive transcriptomic analysis following *Bb* infection could provide new insights into the pathogenesis of LNB and may identify novel biomarkers or therapeutic targets for LNB diagnosis and treatment.

Methods

In the present study, we pooled transcriptomic datasets (transcriptomic rhesus data from our laboratory and the GSE85143 dataset from the Gene Expression Omnibus database) to screen common differentially expressed genes (DEGs) in the *Bb* infection group and the control group. Functional and enrichment analyses were conducted using the Database of Annotation Visualization and Integrated Discovery database, Protein-Protein Interaction network, and hub genes were identified using the Search Tool for the Retrieval of Interaction Genes database and the CytoHubba plugin. In addition, *in vitro* and *ex vivo* assays were performed to verify the above findings. The mRNA expression levels of these genes were verified by quantitative real-time PCR (qPCR).

Results

A total of 80 upregulated DEGs and 32 downregulated DEGs were identified. Among them, 11 hub genes were selected. Upregulated genes in the Gene Ontology analysis were significantly enriched in cell adhesion processes. The pathway enrichment analyses revealed that the PI3K-Akt signaling pathway was significantly enriched. The mRNA levels of *ANGPT1*, *TLR6*, *SREBF1*, *LDLR*, *TNC*, and *ITGA2* in U251 cells and/or rhesus brain explants by exposure to *Bb* were validated by qPCR.

Conclusion

Our study suggested that *TLR6*, *ANGPT1*, *LDLR*, *SREBF1*, *TNC*, and *ITGA* were differentially highly expressed in *Bb*-infected astrocytes compared to normal controls, and overexpression of *LDLR* might be a favorable prognostic factor of LNB patients. Further study is needed to explore the value of *TLR6*, *ANGPT1*, *LDLR*, *SREBF1*, *TNC*, and *ITGA* in LNB pathogenesis.

Introduction

Lyme disease (also known as Lyme borreliosis), mainly caused by *Borrelia burgdorferi* (*Bb*) spirochetes, is the most common vector-borne disease in the northern hemisphere. While frequently occurring in the United States, it is also endemic in Europe and parts of Asia (Schotthoefer and Frost 2015). It has showed increasing growth and expansion in geographic extent over the past two decades, with an estimated 300,000 people infected with Lyme spirochetes annually (Hinckley et al. 2014, Kugeler et al. 2015, Nawrocki and Hinckley 2020). Lyme disease is a multistage and multisystem disorder predominantly affecting the skin, but it also involves the joints, heart, and nervous system. Transmitted by *Ixodes* ticks, its causative agent *Bb* initially propagates locally in the skin before it disseminates hematogenously to other organ systems. Lyme disease which results in neurological manifestation, such as painful meningoradiculitis (Bannwarth syndrome), cranial neuritis, lymphocytic meningitis, and rarely encephalitis or cerebral vasculitis at a later period (stage 2), is called Lyme neuroborreliosis (LNB) (Reik et al. 1979, Stanek et al. 2012). *Bb* is a highly neurophilic pathogen which remains latent in the central or peripheral nervous system, and the clinical presentation varies with disease stages. It has been reported that the incidence of LNB in Lyme disease patients is not less than 10% (Koedel et al. 2015, Steere et al. 2016).

Pain is generally the first neurological symptom of meningoradiculitis, and approximately one-quarter of LNB patients will show no other symptoms. More than half of patients with meningoradiculitis also show cranial nerve symptoms, with unilateral or bilateral facial nerve involvement being the most common (Garcia-Monco and Benach 2019, Hansen and Lebech 1992). Neurological symptoms occur concomitantly with lymphocytic cerebrospinal fluid (CSF) pleocytosis (10–1000 cells per μL), which is a diagnostic criterion for definite LNB in Europe (Mygland et al. 2010, Pfister et al. 1984). Intravenous antibiotics have been proven to be an effective therapy for LNB, however, in a few patients *Bb* has been reported to persist in the CSF after antibiotic therapy (Preac-Mursic et al. 1989). Moreover, a proportion of patients still have symptoms following appropriate antibiotic therapy, which are defined as post-treatment Lyme disease syndrome (Kaplan et al. 2003, Koedel, Fingerle and Pfister 2015). Atypical symptoms and the lack of clinically specific diagnostic indicators often prolongs the diagnosis of LNB or leads to its misdiagnosis. If LNB is left untreated or is improperly treated, neurological symptoms of patients can persist for many years (Feder et al. 2007). Nonetheless, the molecular mechanisms associated with the pathogenesis of LNB remain poorly understood.

In recent years, high-throughput sequencing and bioinformatics technologies have rapidly becoming critical tools in the study of diseases, including Lyme disease (Bouquet et al. 2016, Ding et al. 2020, Mansfield et al. 2017, Petzke et al. 2020). In particular, the study of transcriptomics is more economical and informative than genomics. Transcriptome sequencing of mRNA using high-throughput sequencing technologies can quickly and comprehensively obtain information on almost all transcripts of a specific species tissue or cell in a certain state, including base sequences and gene expressions. Although mRNA only comprises approximately 4% of the total RNA in mammalian cells, it has been the focus of research, because it is ultimately translated into protein and participates in the expression of the cell phenotype (Wu et al. 2014). In addition, the Gene Expression Omnibus (GEO) database developed by the National Center for Biotechnology Information (NCBI) also provides vast valuable datasets.

At present, however, there are limited transcriptomic studies associated with Lyme spirochete infection to CNS. Thus, we conducted this study to explore the pathogenesis of LNB and to provide valuable clues for future research. We obtained the transcriptome data of frontal cortex brain explants that were exposed to *Bb* and the human astrocytes following *Bb* infection transcriptome dataset (GSE85143), and screened the data using the GEO database for comprehensive analysis (Fig. 1). The results of the present study will improve our understanding of the underlying mechanisms of LNB and will identify biomarker candidates for LNB diagnosis and prognosis.

Materials And Methods

Data resources

To identify the host candidate hub genes in models of LNB, we comprehensively analyzed our previous transcriptome raw sequencing data (Ding et al. 2019) which has been uploaded to NCBI in the format of .fastq (<http://www.ncbi.nlm.nih.gov/bioproject/723671>) and the GSE85143 dataset from the GEO database (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) (Clough and Barrett 2016). Frontal cortex brain explants from three healthy rhesus macaques (2 males and 1 female) were used for *ex vivo* experiments in our previous study (Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019, Ding, Sun, Bi, Zhang, Yue, Xu, Cao, Luo, Chen, Li, Ji, Jian, Lu, Abi, Liu and Bao 2020), the explants were collected for transcriptomic analysis after co-culture with live *Bb* (*Bb*4680, DSMZ, A1511358-1) or culture containing normal medium as controls. The platform used for this dataset was Illumina HiSeq 2500. We referred to this data as 'Rhesus dataset' in this paper. The GSE85143 dataset was based on GPL11154 Illumina HiSeq 2000 (*Homo sapiens*) platform. The experiment contained 9 samples consisting of 6 *Bb*-infected samples and 3 uninfected samples, which used human astrocytes infected with *Bb* and uninfected astrocytes as controls.

Differential Expression Analyses

Differential expression analysis was performed using the online analysis tool BioJupies in the GSE85143 dataset. BioJupies (<https://amp.pharm.mssm.edu/biojupies/>) is an easy-to-use web application for RNA-seq data analyses, the datasets published in GEO can be processed by ARCHS4 directly (Torre et al. 2018). The cut-off criteria to select the differentially expressed genes (DEGs) were the adjusted P-value <0.05 and fold-change (FC) ≥2. For the GSE85143 dataset, the volcano plot was visualized using R package ggplot2. The differential analysis of the Rhesus dataset has been described in detail in our previous study (Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019). We merged all DEGs from three time points in the Rhesus dataset. Statistical analysis was performed for each dataset using the Venn diagram webtool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to identify the intersecting DEGs.

Functional and Pathway Enrichment Analyses

The Gene ontology (GO) term enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs were performed using DAVID (the Database for Annotation, Visualization, and Integration Discovery). DAVID (version 6.8, <https://david.ncifcrf.gov/>) is a web server which provides a comprehensive set of functional annotation tools to help researchers understand the biological significance of a large list of genes (Huang et al. 2007). GO is an important biological tool that enables investigators to turn data into knowledge. GO comprises three categories including Biological process (BP), Molecular function (MF), and Cellular component (CC) (Ashburner et al. 2000). A P-value of <0.05 was used as the threshold for significance.

Protein–Protein Interaction Network Analysis and Identification of Hub Genes

The protein–protein interaction (PPI) network was analyzed and visualized using the online resource of Search Tool for the Retrieval of Interaction Genes (STRING) 11.0 (<https://string-db.org/>), which collects, scores, and integrates functional interactions among proteins (Szklarczyk et al. 2019). We mapped previously identified DEGs to STRING to identify potential interactions between them. In our study, the minimum interaction score required was 0.4 (medium confidence). The PPI network was visualized by Cytoscape 3.8.2 and analyzed in Cytoscape software with the cytoHubba plugin. Eleven hub genes closely linked to models of LNB were identified using MCC, DMNC, MNC, Degree, EPC, Bottleneck, Eccentricity, Closeness, Radiality, Betweenness, Stress, Clustering Coefficient methods. Multi-dimensional Venn diagrams were generated and visualized using the R package UpSetR (Conway et al. 2017).

Spirochetes Culture

Borrelia burgdorferi sensu stricto strain 4680 (DSMZ, A1511358-1) culture was carried out as described previously with little modification (Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019). For co-culture experiments, spirochetes were centrifuged (2500×g; 10 min; 4°C) and the Barbour-Stoenner-Kelly II (BSK II) medium supplemented with 6% rabbit serum (Thermo Fisher Scientific, United States) was removed and washed three times with sterilized PBS. The spirochetes were resuspended in antibiotic-free cell culture medium. Bacterial counts were determined using a counting plate under dark-field microscopy.

Co-culture of the Human Astrocyte Cell Line with *Bb*

The human astrocyte cell line U251 was obtained from the Kunming Cell Bank, Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). U251 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, United States) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, United States) at 37°C with 5% CO₂. U251 cells were seeded in 6-well plates at a cell density of 1.2×10⁵ cells per well. After attaching to plates, cells were exposed to *Bb* at a multiplicity of infection (MOI) of 0.1 or 1 for 6 h, 12 h, 24 h, and 48 h.

Co-culture of Frontal Cortex Brain Explants with *Bb*

Fresh rhesus frontal cortex tissue sections were collected during the animal necropsy, and the repeated specimens were co-cultured with live spirochetes as described in our previously published studies(Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019). The brain explants were stored with Trizol reagent (Invitrogen, United States) at -80°C.

RNA Isolation and Reverse Transcription

Total brain RNA was extracted from brain explants by phenol and chloroform extraction as well as isopropanol precipitation, and then washed with ice-cold 75% ethanol. The total cell RNA was extracted from U251 using a Trizol reagent. Total RNA was reverse-transcribed into cDNA using the PrimeScript RTreagent kit with gDNA Eraser (Takara, Japan) according to the manufacturer's instructions. The final reaction mixture volume was 20 µl. Specific details were referenced to our previous work(Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019).

Validation of Result by Quantitative Real-Time Polymerase Chain Reaction (qPCR)

The resulting cDNA was used to perform qPCR on the CFX Connect System (Bio-Rad Laboratories, Inc.). The total qPCR reaction volume was 25 µL and included Takara SYBR Green II (12.5 µL), forward and reverse primers (1 µL of each), RNase-free water (8.5 µL) and cDNA template (2 µL). The cycling conditions were as follows: 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec, and 58.5°C for 30 sec. The sequences of the specific primers used for the above reactions are shown in **Table 1**. Human *β-Actin* and rhesus *GAPDH* were used as the internal references. The relative expression of target genes was calculated with the $2^{-\Delta\Delta C_t}$ method(Livak and Schmittgen 2001).

Statistical Analysis

All data were reported as the mean±standard error of the mean (SEM) and were analyzed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). All experiments were repeated in triplicate. Two-Way ANOVA was applied for group comparisons. A P-value <0.05 was considered statistically significant.

Results

Identification of DEGs between Unexposed and *Bb*-infected Groups

We used the gene expression profiles of the GSE85143 and the Rhesus dataset from our previous study(Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019). In our previous study, we co-cultured rhesus macaques' frontal cortex brain explants with living spirochetes. There were 6 control samples and 6 *Bb* infected samples. The GSE85143 contains 6 *Bb* infected samples and 3 untreated samples. According to the cut-off criteria P-value <0.05 and FC ≥2, from the Rhesus dataset, we identified 3075 DEGs, including 2116 upregulated genes and 959 downregulated genes. Based on the RNA-seq GSE85143 dataset, a total of 1450 DEGs were identified, and comprised 724

upregulated genes and 726 downregulated genes (**Supplemental File1**). For the GSE85143 dataset, the DEGs are shown in the volcano plot and in the heatmap, whose clustering was performed using BioJupies (**Fig 2**). Moreover, the principal component analysis (PCA) was also performed to examine differences between *Bb* infected and untreated groups (**Fig. S1**). All DEGs were defined by comparison of untreated samples and *Bb* infected samples. The Venn diagram showed the intersection of the DEGs in the two sets (**Fig. 3**). The results indicated there was a total of 112 matched DEGs between the two datasets, of which 80 were upregulated genes (**Supplemental File 2**) and 32 were downregulated genes (**Supplemental File 3**).

GO Annotations and KEGG Pathways

The functional annotation analysis and KEGG pathway enrichment analysis of DEGs were performed using web tool DAVID. GO analysis indicated the annotation of DEGs from three categories: BP, CC, and MF (**Fig. 4**). The results revealed that the upregulated DEGs in the BP category were mainly enriched in cell adhesion, negative regulation of endopeptidase activity, single organismal cell-cell adhesion or leukocyte migration, and response to hypoxia. While the downregulated DEGs in the BP were mainly involved in extracellular matrix organization and cell proliferation. CC analysis showed that the upregulated DEGs were significantly enriched in plasma membrane, extracellular region, extracellular space and the cytoskeleton, while the downregulated DEGs in CC were mainly involved in integral components of the membrane, extracellular space, extracellular region, endoplasmic reticulum membrane, and the proteinaceous extracellular matrix. For MF analysis, the upregulated DEGs were mainly enriched in heparin binding and carbohydrate binding, the downregulated DEGs were enriched in extracellular matrix structural constituents and binding. In addition, the upregulated DEGs were significantly mapped on complement and coagulation cascades, hematopoietic cell lineage, *Staphylococcus aureus* infection, and arrhythmogenic right ventricular cardiomyopathy (ARVC) in the KEGG enrichment analysis (**Fig. 5**).

PPI Network Analysis and Identification of Hub genes

To investigate the potential relationships between DEGs, we attempted to construct a PPI network using the STRING database. In total, 117 nodes and 95 edges were presented in this PPI (PPI enrichment P-value was lower than 1.44×10^{-7}) network (**Fig. 6**). The DEGs were comprehensively calculated to identify the intersections among 12 different algorithms, and the most notable 11 genes were considered as hub genes (**Table 2**). The multi-dimensional Venn diagram revealed the intersection of 12 algorithms for DEGs (**Fig. 7A**). The heat map showed the differential expression of hub genes in our Rhesus samples (**Fig. 7B**). A PPI (PPI enrichment P-value $< 2.17 \times 10^{-8}$) network of the hub genes was constructed using STRING, a total of 11 nodes and 13 edges were involved (**Fig. 7C**). Functional analysis of hub genes was performed using DAVID. The results showed that the hub genes were mainly enriched in the extracellular matrix organization, plasma membrane, and receptor activity (**Table 3**). The KEGG pathway analysis showed the hub genes were significantly enriched in PI3K-Akt signaling pathway (**Table 3**).

Validation of Hub Genes *in vitro*

Among the identified hub genes *SELP*, *CD93*, *ANGPT1*, *TLR6*, and *SERPIND1* were upregulated, while *SREBF1*, *LDLR*, *MATN3*, *TNC*, *ITGA2*, and *CSF1R*, were downregulated. To verify these results, we conducted validation experiments using qPCR *in vitro*. The U251 cells were co-cultured with live *Bb* for 6 h, 12 h, 24 h, and 48 h at MOI of 0.1 or 1, and cells cultured in normal medium served as control. Subsequently, total RNA was isolated and qPCR was performed as mentioned above. For upregulated hub genes, the mRNA level of *TLR6* and *ANGPT 1* were significantly upregulated in both MOI=0.1 and MOI=1 groups as compared to the controls (**Fig. 8A, B**). For downregulated hub genes, we verified that *LDLR*, *SREBF 1*, *TNC*, and *ITGA*, presented a downward trend at different time points after cells were stimulated by live *Bb* (**Fig. 8C-F**). Based on the above results, we speculated that the hub genes *TLR6*, *ANGPT 1*, *LDLR*, *SREBF1*, *TNC*, and *ITGA2* were potentially associated with the pathogenesis of LNB.

Validation of Hub Genes in *ex vivo*

To further verify the expression of these hub genes, we also verified their expression in the *ex vivo* model by qPCR. We found that the mRNA expression level of *TLR6* in the *Bb*-infected group was upregulated compared to the untreated group, especially at the 24 h timepoint (**Fig. 9A**). For downregulated hub genes, the results showed that the mRNA expression level of *LDLR* in the *Bb*-infected group had decreased compared with the control group ($P = 0.08$) at 12 h, whereas no significant differences were observed at the other two timepoints (**Fig. 9B**). In the *Bb* group, the mRNA expression level of *TNC* showed a downward trend over time, however the results were not statistically significant (**Fig. 9C**). These results indicated that *TLR6* was most likely a critical molecule involved in the pathogenesis of LNB.

Discussion

LNB is a nervous system disorder caused by spirochete *Bb* infection. At present, the efficacy of antibiotics in the treatment of LNB has been defined. Early detection and prompt anti-pathogen treatment can improve the prognosis of patients (Rauer et al. 2018). However, due to the lack of specific clinical manifestations, sporadic cases in non-endemic areas are usually missed and often misdiagnosed. Patients with LNB can experience serious consequences such as dementia or personality disorders in the absence of timely treatment, which greatly impacts on their quality of life. Therefore, it is crucial to study the pathogenic biomarkers of LNB that may provide evidence for early diagnosis and treatment.

Recently, bioinformatics analysis has become the conventional means for medical research into disease diagnosis or treatment, and it has significantly accelerated the utilization and integration of public biomedical resources. Nonetheless, to date, only a few omics studies have pointed to models of LNB. Our previous study established a dataset of the transcriptional changes induced by *Bb* in fresh rhesus frontal cortex tissues (Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019). In the present study, for the first time we conducted a comprehensive analysis of transcriptional mRNA changes induced by *Bb* both in rhesus brain tissue and primary human astrocytes (Casselli et al. 2017). Similarly, DEGs from astrocytes co-cultured with *Bb* and control astrocytes were identified and integrated with the gene expression dataset GSE85143 from the GEO database.

Since the two datasets generated on different sequencing platforms (Illumina HiSeq 2500 & Illumina HiSeq 2000), the DEGs associated with Lyme spirochete infection to CNS were identified by taking the intersection analysis on each dataset. Some original data used in the current study have been used in prior publications, however, we generated a set of DEGs by taking the union from all time points. The sample species are different between our rhesus dataset and GSE85143, the DAVID Gene ID Conversion Tool was used. After deleting duplicate and invalid genes, we finally identified 3,075 differentially expressed genes in this study. In our previous study, the number of DEGs identified were 2,249 (6 h post-exposure), 1,064 (12 h post-exposure), and 420 (24 h post-exposure)(Ding, Ma, Tao, Peng, Han, Sun, Dai, Ji, Bai, Jian, Chen, Luo, Wang, Bi, Liu and Bao 2019). In the study conducted by Casselli *et al.*, GSE85143 is a part of superseries, GSE85143 is RNA-seq dataset which reveals changes in the astrocyte transcriptome following *Bb* infection and the other parts were about non-coding RNA profiling. We only focused on the analysis of mRNA expression profile for comprehensive analysis. We used the samples infected with *Bb* at 24 h and 48 h as test group and the three uninfected as control. Accordingly, there are 1450 DEGs in GSE85143 which could be compared to previously published results(Casselli, Qureshi, Peterson, Perley, Blake, Jokinen, Abbas, Nechaev, Watt, Dhasarathy and Brisette 2017).

Herein, we screened and identified 80 upregulated DEGs (**Supplemental File 2**) and 32 downregulated DEGs (**Supplemental File 3**). Among the upregulated DEGs, most GO terms in the BP group were associated with cell adhesion, while for downregulated DEGs, the significantly enriched transcripts in BP GO terms were related to extracellular matrix organization. The KEGG pathway analysis indicated that these upregulated DEGs were significantly enriched in pathways involved in the complement and coagulation cascades, hematopoietic cell lineage, *Staphylococcus aureus* infection and ARVC. There were no significantly enriched KEGG pathways associated with downregulated DEGs. We constructed a PPI network by screening 11 hub genes. To obtain more reliable results, the 112 DEGs of 12 algorithms were investigated using the CytoHubba plugin. The results of top 30 DEGs of each algorithm were sorted by rank and the following intersection genes were considered as hub genes (**Supplemental File 4**): *SREBF1*, *LDLR*, *SELP*, *CD93*, *ANGPT1*, *TLR6*, *MATN3*, *SERPIND1*, *TNC*, *ITGA2*, and *CSF1R*. Among these hub genes, *SELP*, *CD93*, *ANGPT1*, *TLR6*, and *SERPIND1* were upregulated, while *SREBF1*, *LDLR*, *MATN3*, *TNC*, *ITGA2*, and *CSF1R* were downregulated. Furthermore, we performed KEGG pathway enrichment analyses on hub genes and the results revealed that the PI3K-Akt signaling pathway showed the most significant difference (P-value < 0.01). Finally, we validated these results using *ex vivo* samples of rhesus frontal cortex brain explants and *in vitro* in human U251 cell lines using qPCR at the transcription level. These results suggested that *TLR6*, *ANGPT1*, *SREBF1*, *LDLR*, *TNC*, and *ITGA2* might be involved in the pathogenesis of LNB.

TLR6 is a member of the Toll-like receptor (TLR) family, which plays a key role in pathogen-associated molecular patterns (PAMPs) recognition and in innate immune responses. TLR1, TLR2, and TLR6 are all located on the cell membrane. They share high protein homology in their transmembrane and cytoplasmic regions, while their extracellular region is diverse. Thus, they can recognize specific pathogens and activate common intracellular signaling pathways(Takeuchi et al. 1999). It has been shown that TLR is a driving force behind *Bb* infection, and that the TLR signaling pathway plays an

important role in inducing inflammatory responses(Koenig et al. 2009, Singh and Girschick 2006, Thomas and Fikrig 2002). Specifically, TLR2 plays an important role in the recognition of Lyme spirochetes(Hirschfeld et al. 1999). TLR6 forms heterodimer with TLR2 and distributes along the cell surface to specifically recognize diacylated lipopeptides and partly triacylated lipopeptides(Kang et al. 2009). A previous study showed that blocking TLR6 on human peripheral blood cells after exposure to *Bb* did not reduce the production of cytokines, while blocking TLR1 significantly decreased cytokine production(Oosting et al. 2011). Further, the *Bb*-induced secretion of IFN- γ in murine cells depends on TLR6(Oosting, Ter Hofstede, Sturm, Adema, Kullberg, van der Meer, Netea and Joosten 2011). Our previous studies have also shown that the TLR1/TLR2 ratio plays an important role in initiating of proinflammatory chemokine storm in Lyme disease(Zhao et al. 2019). However, due to gene expression specificities in different species, tissues or cell lineages, the role of *TLR6* in LNB needs to be further explored. In the present study, *TLR6* is the only hub gene that was verified to be elevated in both the human U251 cell line and in rhesus brain explants when exposed to *Bb*. The transcription level of *TLR6* began to increase at 12 h after human astrocytes were exposed to *Bb*, and the increased expression was observed at only 6 h after rhesus brain explants were exposed to *Bb*. This may be because other immune cells in the brain tissue, such as microglia, are also involved in the pathophysiological process in the early stages of LBN. Additionally, further analysis of KEGG enrichment analysis of hub genes suggested that the PI3K-AKT pathway may play a key role in the pathogenesis of LNB. Interestingly, we found that the TLR6 signals could activate this pathway using the KEGG pathway mapping tool (**Fig. S2**). Therefore, *TLR6* may take part in the pathogenesis of LNB.

With regard to the upregulated hub genes, in addition to TLR6, another gene that has been verified to be upregulated in human astrocytes exposed to *Bb* is *ANGPT1*. This result is consistent with the results of the study by Casselli *et al.*(Casselli, Qureshi, Peterson, Perley, Blake, Jokinen, Abbas, Nechaev, Watt, Dhasarathy and Brissette 2017). They confirmed the elevation of angiopoietin-like 4 (ANGPTL4) in human primary astrocytes exposed to *Bb* by enzyme-linked immunosorbent assays. Both *ANGPT1* and *ANGPTL4* belong to the angiopoietin family and encode secreted glycoproteins that play critical roles in development and in disorders including in angiogenesis, inflammation, cell proliferation, apoptosis, lipid and glucose metabolism, cell migration, and cancer(Carbone et al. 2018, Ehrlich et al. 2019, Snipstad et al. 2010, Zhang et al. 2018). Compared with other ANGPT/ANGPTL genes which are embedded in the introns of larger genes, *ANGPT1* and *ANGPTL4* are stand-alone genes whose expression correlated with tissue-specific enhancer or promoter chromatin sequences(Ehrlich, Lacey and Ehrlich 2019). *ANGPT1* is the ligand of the transmembrane tyrosine kinase TIE2 receptor. Phosphorylation of tyrosine kinase can activate the PI3K/AKT pathway (**Fig. S3**), which was a core signaling pathway identified in our previous studies(Fukuhara et al. 2008, Huang et al. 2010). *ANGPT2*, which is highly homologous to *ANGPT1*, has a completely different effect on TIE2(Parikh 2017). The involvement of *ANGPT1* and *ANGPT2* in pathological inflammation is clear. In several diseases that have a strong association with inflammation, such as sepsis and malignant tumors, angiopoietins can be used as prognostic indicators(Fiedler and Augustin 2006, Park et al. 2009, Seol et al. 2020). In our previous study(Zhao et al. 2018), we found that the NF- κ B pathway plays a key role in the pathogenesis of LNB, and other studies have reported that

ANGPT can also influence the NF- κ B pathway(Fiedler and Augustin 2006, Huang, Bhat, Woodnutt and Lappe 2010). These results suggested the possible potential of *ANGPT* either as novel diagnostic and prognostic indicators, or as a therapeutic target for LNB. In addition, upregulation of *SERPIN* in the upregulated hub genes were verified in the study by Casselli *et al*(Casselli, Qureshi, Peterson, Perley, Blake, Jokinen, Abbas, Nechaev, Watt, Dhasarathy and Brissette 2017). Thus, they have not been discussed in detail here.

Among the downregulated hub genes, we verified that the expression of *LDLR*, *SREBF1*, *TNC*, and *ITGA* showed a statistically significant decrease in human astrocytes on exposure to *Bb*. Following exposure of rhesus brain explants to *Bb*, we found there was a decrease in both *LDLR* and *TNC* levels, although there the differences were not statistically significant (P-values of *LDLR* at 6 h, 12 h and 24 h *Bb*-exposed astrocytes vs controls were 0.8, 0.08, and 0.37, respectively). *LDLR* is a cell surface glycoprotein that can be expressed on various cell types including astrocytes in many tissues. *LDLR* can specifically recognize and bind lipoproteins containing apolipoprotein (Apo)E or ApoB100 to mediate cholesterol metabolism, thus it is a central component for the maintenance of cholesterol homeostasis(Brown and Goldstein 1986). Studies have elucidated that LNB may promote the neuro-inflammatory response and endow susceptibility to Alzheimer's disease (AD), which can not only lead to dementia, but also may induce pathological features typical of AD, such as amyloid-beta ($A\beta$) deposits(Miklossy et al. 2004, Miklossy et al. 2006). Conversely, upregulating the expression of *LDLR* can improve the brain clearance of $A\beta$, reduce amyloid-deposition and attenuate the neuroinflammatory response(Basak et al. 2012, Yao et al. 2016). A recent study revealed that lower *LDLR* expression can aggravate the neuronal inflammatory response, which might occur through NF- κ B signaling and NLRP3-ASC caspase-1 inflammasome assembly(Sun et al. 2020). Thus, *LDLR* may serve as a protective factor for LNB.

Meanwhile, the reduction of *SREBF1* expression at the transcriptional level has been verified in human U251 cells at exposure to *Bb*. The proteins encoded by *SREBFs* are transcription factors that control cholesterol homeostasis. *LDLR* is one of the target genes regulated by sterol regulatory element-binding proteins (SREBPs)(Horton et al. 2003). We observed a decrease in *TNC* transcriptional expression of U251 cells after stimulation by *Bb*, and we also found a downward trend over time in rhesus brain explants, albeit there was no statistically significant difference. The protein encoded by *TNC* belongs to the tenascin family and is widely involved in pathological processes such as inflammation and malignancy. *TNC* plays a key role in the proliferation of primary astrocytes(Ikeshima-Kataoka et al. 2008, Roll and Faissner 2019). There have been many reports that *TNC* is highly expressed when the CNS is injured(Roll and Faissner 2019). However, brain injured *TNC* knock-out mice showed higher expression of inflammatory factors such as TNF- α , IL-6, and IL-1 β than wild-type mice(Ikeshima-Kataoka, Shen, Eto, Saito and Yuasa 2008). The expression level of *TNC* varies under different pathological conditions. It may help regulate the production of inflammatory factors in the damaged brain. In addition, *ITGA2* reduction was observed at 6 h and 48 h in U251 cells after exposure to *Bb*. *ITGA2* is a glycoprotein of the integrin family, which mediates cell-cell interactions and those of the cell-extracellular matrix. It also participates in the pathophysiological process of inflammation and the immune response. However, studies have reported that *ITGA2* can aggravate the destruction of inflammatory cartilage in rheumatoid

arthritis, and has been implicated in cell growth and apoptosis in tumors (Penrose et al. 2019, Peters et al. 2012). The roles of ITGA2 in LNB remain to be clarified, therefore, further studies are needed.

All in all, in present study, we combined two different datasets related to LNB and identified a group of commonly affected transcripts. Using the commonly affected genes from two very different experimental systems to conduct detailed functional and network analyses. Among them, 11 hub genes were identified from network analysis. We also conducted validation experiments and validated some of the hub genes.

There are some limitations in our current study. First, *in vivo* experimental validation is needed to validate these findings. In addition, although we have verified the mRNA expression of hub genes using a cell line *in vitro* and in *ex vivo* rhesus brain explants, we did not carry out in-depth mechanism research. Additionally, due to the different sample sources and species of the two datasets, the intersection of the DEGs could provide only limited information. Moreover, the sufficient validation by alternate methods or by measuring the protein levels for biological relevance to LNB will need to be performed in future work. And the number of samples is relatively small. The underlying mechanisms need to be further studied in our future studies.

In conclusion, the present study suggests that *TLR6*, *ANGPT1*, *LDLR*, *SREBF1*, *TNC*, and *ITGA* were differentially highly expressed in *Bb*-infected astrocytes compared to control astrocytes. These candidate genes are clinically promising biomarkers that can be used for LNB diagnosis or treatment. In addition, *LDLR* was found to be significantly associated with favorable outcomes in neuroinflammatory diseases, which may provide a better understanding of molecular mechanisms and novel targets as therapeutic strategies in the future. Thus, our findings may provide valuable insights into the study of LNB pathogenesis and provides guidance for designing further follow-up studies.

Declarations

a

ETHICAL STATEMENT:

Ethics approval and consent to participate

This experiment was performed according to the Guide for the Care and Use of Laboratory Animals and ARRIVE Guidelines for Reporting Animal Research. The Animal Ethics and Welfare Committee of Kunming Medical University reviewed and approved this study. The animal permit number: SCXK (DIAN) K2015-0004.

Consent for publication

Not applicable.

Availability of data and materials

All data files described in this manuscript for analysis, are available at the NCBI site:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE85143>

<http://www.ncbi.nlm.nih.gov/bioproject/723671>

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 32060180, 81860644, 81560596, and 31560051) and the Natural Foundation of Yunnan Province [No. 2019FE001(-002) and 2017FE467(-001)]. The funding institutions had no involvement in the design of the study or review of the manuscript.

Authors' contributions

All authors contributed to the study conception and design. Fukai Bao, Aihua Liu, and Shiyuan Wen conceived and designed the experiments. Shiyuan Wen, Xin Xu, Jing Kong, Lisha Luo, Yu Zhang, Mingbiao Ma, Lvyan Tao and Yun Peng developed the methodology. Shiyuan Wen, Xin Xu, Wenjing Cao, Peng Yue, Yuxin Fan, Meixiao Liu and Jingjing Chen performed all experiments. Yan Dong, Suyi Luo, Bingxue Li, Feng Wang, Guozhong Zhou, Taigui Chen and Lianbao Li analyzed and discussed the data. Shiyuan Wen wrote the manuscript. Shiyuan Wen, Fukai Bao and Aihua Liu edited and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the Yunnan Province Key Laboratory for Tropical Infectious Diseases in Universities; Yunnan Province Integrative Innovation Center for Public Health, Diseases Prevention and Control; Kunming Medical University; and Yunnan Demonstration Base of International Science and Technology Cooperation for Tropical Diseases (all located in Kunming, China) for supporting this study.

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Acknowledgements

The authors thank the Yunnan Province Key Laboratory for Tropical Infectious Diseases in Universities; Yunnan Province Integrative Innovation Center for Public Health, Diseases Prevention and Control; Kunming Medical University; and Yunnan Demonstration Base of International Science and Technology Cooperation for Tropical Diseases (all located in Kunming, China) for supporting this study.

References

1. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT et al (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* May 25:25–29. Epub 2000/05/10
2. Basak JM, Verghese PB, Yoon H, Kim J, Holtzman DM (2012) Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of Abeta uptake and degradation by astrocytes. *J Biol Chem* Apr 20:287:13959–13971. Epub 2012/03/03
3. Bouquet J, Soloski MJ, Swei A, Cheadle C, Federman S, Billaud JN, Rebman AW, Kabre B, Halpert R, Boorgula M et al (2016) Longitudinal Transcriptome Analysis Reveals a Sustained Differential Gene Expression Signature in Patients Treated for Acute Lyme Disease. *mBio*. Feb 12;7:e00100-00116. Epub 2016/02/14
4. Brown MS, Goldstein JL (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* Apr 4:232:34–47. Epub 1986/04/04
5. Carbone C, Piro G, Merz V, Simionato F, Santoro R, Zecchetto C, Tortora G, Melisi D (2018) Angiopoietin-Like Proteins in Angiogenesis, Inflammation and Cancer. *Int J Mol Sci*. Feb 1;19. Epub 2018/02/02
6. Casselli T, Qureshi H, Peterson E, Perley D, Blake E, Jokinen B, Abbas A, Nechaev S, Watt JA, Dhasarathy A et al (2017) MicroRNA and mRNA Transcriptome Profiling in Primary Human Astrocytes Infected with *Borrelia burgdorferi*. *PLoS One* 12:e0170961. Epub 2017/01/31
7. Clough E, Barrett T (2016) The Gene Expression Omnibus Database. *Methods Mol Biol* 1418:93–110. Epub 2016/03/24
8. Conway JR, Lex A, Gehlenborg N (2017) UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* Sep 15:33:2938–2940. Epub 2017/06/25
9. Ding Z, Ma M, Tao L, Peng Y, Han Y, Sun L, Dai X, Ji Z, Bai R, Jian M et al (2019) Rhesus Brain Transcriptomic Landscape in an ex vivo Model of the Interaction of Live *Borrelia burgdorferi* With Frontal Cortex Tissue Explants. *Frontiers in Neuroscience*.13
10. Ding Z, Sun L, Bi Y, Zhang Y, Yue P, Xu X, Cao W, Luo L, Chen T, Li L et al (2020) Integrative Transcriptome and Proteome Analyses Provide New Insights Into the Interaction Between Live *Borrelia burgdorferi* and Frontal Cortex Explants of the Rhesus Brain. *J Neuropathol Exp Neurol* May 1:79:518–529. Epub 2020/03/21
11. Ehrlich KC, Lacey M, Ehrlich M (2019) Tissue-specific epigenetics of atherosclerosis-related ANGPT and ANGPTL genes. *Epigenomics* Feb 11:169–186. Epub 2019/01/29
12. Feder HM Jr, Johnson BJ, O'Connell S, Shapiro ED, Steere AC, Wormser GP, Ad Hoc International Lyme Disease G, Agger WA, Artsob H, Auwaerter P et al (2007) A critical appraisal of "chronic Lyme disease". *N Engl J Med* Oct 4:357:1422–1430. Epub 2007/10/05
13. Fiedler U, Augustin HG (2006) Angiopoietins: a link between angiogenesis and inflammation. *Trends Immunol* Dec 27:552–558. Epub 2006/10/19
14. Fukuhara S, Sako K, Minami T, Noda K, Kim HZ, Kodama T, Shibuya M, Takakura N, Koh GY, Mochizuki N (2008) Differential function of Tie2 at cell-cell contacts and cell-substratum contacts

- regulated by angiopoietin-1. *Nat Cell Biol* May 10:513–526. Epub 2008/04/22
15. Garcia-Monco JC, Benach JL (2019) Lyme Neuroborreliosis: Clinical Outcomes, Controversy, Pathogenesis, and Polymicrobial Infections. *Ann Neurol* Jan 85:21–31. Epub 2018/12/12
 16. Hansen K, Lebech AM (1992) The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985–1990. A prospective study of 187 patients with *Borrelia burgdorferi* specific intrathecal antibody production. *Brain* Apr 115(Pt 2):399–423. Epub 1992/04/01
 17. Hinckley AF, Connally NP, Meek JI, Johnson BJ, Kemperman MM, Feldman KA, White JL, Mead PS (2014) Lyme disease testing by large commercial laboratories in the United States. *Clin Infect Dis* Sep 1:59:676–681. Epub 2014/06/01
 18. Hirschfeld M, Kirschning CJ, Schwandner R, Wesche H, Weis JH, Wooten RM, Weis JJ (1999) Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J Immunol* Sep 1:163:2382–2386. Epub 1999/08/24
 19. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, Goldstein JL (2003) Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc Natl Acad Sci U S A* Oct 14:100:12027–12032. Epub 2003/09/27
 20. Huang DW, Sherman BT, Tan Q, Kir J, Liu D, Bryant D, Guo Y, Stephens R, Baseler MW, Lane HC et al (2007) DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res* Jul 35:W169–W175. Epub 2007/06/20
 21. Huang H, Bhat A, Woodnutt G, Lappe R (2010) Targeting the ANGPT-TIE2 pathway in malignancy. *Nat Rev Cancer* Aug 10:575–585. Epub 2010/07/24
 22. Ikeshima-Kataoka H, Shen JS, Eto Y, Saito S, Yuasa S (2008) Alteration of inflammatory cytokine production in the injured central nervous system of tenascin-deficient mice. *Vivo* Jul-Aug 22:409–413. Epub 2008/08/21
 23. Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO (2009) Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. *Immunity* Dec 18:31:873–884. Epub 2009/11/26
 24. Kaplan RF, Trevino RP, Johnson GM, Levy L, Dornbush R, Hu LT, Evans J, Weinstein A, Schmid CH, Klempner MS (2003) Cognitive function in post-treatment Lyme disease: do additional antibiotics help? *Neurology* Jun 24:60:1916–1922. Epub 2003/06/25
 25. Koedel U, Fingerle V, Pfister HW (2015) Lyme neuroborreliosis-epidemiology, diagnosis and management. *Nat Rev Neurol* Aug 11:446–456. Epub 2015/07/29
 26. Koenig CL, Miller JC, Nelson JM, Ward DM, Kushner JP, Bockenstedt LK, Weis JJ, Kaplan J, De Domenico I (2009) Toll-like receptors mediate induction of hepcidin in mice infected with *Borrelia burgdorferi*. *Blood* Aug 27:114:1913–1918. Epub 2009/07/10
 27. Kugeler KJ, Farley GM, Forrester JD, Mead PS (2015) Geographic Distribution and Expansion of Human Lyme Disease, United States. *Emerg Infect Dis* Aug 21:1455–1457. Epub 2015/07/22
 28. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* Dec 25:402–408. Epub 2002/02/16

29. Mansfield KL, Cook C, Ellis RJ, Bell-Sakyi L, Johnson N, Alberdi P, de la Fuente J, Fooks AR (2017) Tick-borne pathogens induce differential expression of genes promoting cell survival and host resistance in *Ixodes ricinus* cells. *Parasit Vectors* Feb 15:10:81. Epub 2017/02/17
30. Miklosy J, Khalili K, Gern L, Ericson RL, Darekar P, Bolle L, Hurlimann J, Paster BJ (2004) *Borrelia burgdorferi* persists in the brain in chronic lyme neuroborreliosis and may be associated with Alzheimer disease. *J Alzheimers Dis* Dec 6:639–649; discussion 673–681. Epub 2005/01/25
31. Miklosy J, Kis A, Radenovic A, Miller L, Forro L, Martins R, Reiss K, Darbinian N, Darekar P, Mihaly L et al (2006) Beta-amyloid deposition and Alzheimer's type changes induced by *Borrelia* spirochetes. *Neurobiol Aging* Feb 27:228–236. Epub 2005/05/17
32. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I, European Federation of Neurological S (2010) EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. *Eur J Neurol* Jan 17:8–16, e11-14. Epub 2009/11/26
33. Nawrocki CC, Hinckley AF (2020) Experiences with tick exposure, Lyme disease, and use of personal prevention methods for tick bites among members of the U.S. population, 2013–2015. *Ticks Tick Borne Dis* Nov 2:12:101605. Epub 2020/11/21
34. Oosting M, Ter Hofstede H, Sturm P, Adema GJ, Kullberg BJ, van der Meer JW, Netea MG, Joosten LA (2011) TLR1/TLR2 heterodimers play an important role in the recognition of *Borrelia* spirochetes. *PLoS One* 6:e25998. Epub 2011/10/15
35. Parikh SM (2017) Angiopoietins and Tie2 in vascular inflammation. *Curr Opin Hematol* Sep 24:432–438. Epub 2017/06/06
36. Park JH, Choi H, Kim YB, Kim YS, Sheen SS, Choi JH, Lee HL, Lee KS, Chung WY, Lee S et al (2009) Serum angiopoietin-1 as a prognostic marker in resected early stage lung cancer. *Lung Cancer* Dec 66:359–364. Epub 2009/04/03
37. Penrose HM, Cable C, Heller S, Ungerleider N, Nakhoul H, Baddoo M, Hartono AB, Lee SB, Burow ME, Flemington EF et al (2019) Loss of Forkhead Box O3 Facilitates Inflammatory Colon Cancer: Transcriptome Profiling of the Immune Landscape and Novel Targets. *Cell Mol Gastroenterol Hepatol* 7:391–408. Epub 2019/02/06
38. Peters MA, Wendholt D, Strietholt S, Frank S, Pundt N, Korb-Pap A, Joosten LA, van den Berg WB, Kollias G, Eckes B et al (2012) The loss of alpha2beta1 integrin suppresses joint inflammation and cartilage destruction in mouse models of rheumatoid arthritis. *Arthritis Rheum* May 64:1359–1368. Epub 2011/11/16
39. Petzke MM, Volyanskyy K, Mao Y, Arevalo B, Zohn R, Quituisaca J, Wormser GP, Dimitrova N, Schwartz I (2020) Global Transcriptome Analysis Identifies a Diagnostic Signature for Early Disseminated Lyme Disease and Its Resolution. *mBio*. Mar 17;11. Epub 2020/03/19
40. Pfister HW, Einhaupl K, Preac-Mursic V, Wilske B, Schierz G (1984) The spirochetal etiology of lymphocytic meningoradiculitis of Bannwarth (Bannwarth's syndrome). *J Neurol* 231:141–144. Epub 1984/01/01

41. Preac-Mursic V, Weber K, Pfister HW, Wilske B, Gross B, Baumann A, Prokop J (1989) Survival of *Borrelia burgdorferi* in antibioticly treated patients with Lyme borreliosis. *Infection* Nov-Dec 17:355–359. Epub 1989/11/01
42. Rauer S, Kastenbauer S, Fingerle V, Hunfeld KP, Huppertz HI, Dersch R (2018) Lyme Neuroborreliosis. *Dtsch Arztebl Int* Nov 9:115:751–756. Epub 2018/12/24
43. Reik L, Steere AC, Bartenhagen NH, Shope RE, Malawista SE (1979) Neurologic abnormalities of Lyme disease. *Medicine (Baltimore)* Jul 58:281–294. Epub 1979/07/01
44. Roll L, Faissner A (2019) Tenascins in CNS lesions. *Semin Cell Dev Biol* May 89:118–124. Epub 2018/10/06
45. Schotthoefer AM, Frost HM (2015) Ecology and Epidemiology of Lyme Borreliosis. *Clin Lab Med* Dec 35:723–743. Epub 2015/11/26
46. Seol CH, Yong SH, Shin JH, Lee SH, Leem AY, Park MS, Kim YS, Chung KS (2020) The ratio of plasma angiopoietin-2 to angiopoietin-1 as a prognostic biomarker in patients with sepsis. *Cytokine* May 129:155029. Epub 2020/02/15
47. Singh SK, Girschick HJ (2006) Toll-like receptors in *Borrelia burgdorferi*-induced inflammation. *Clin Microbiol Infect* Aug 12:705–717. Epub 2006/07/18
48. Snipstad K, Fenton CG, Kjaeve J, Cui G, Anderssen E, Paulssen RH (2010) New specific molecular targets for radio-chemotherapy of rectal cancer. *Mol Oncol* Feb 4:52–64. Epub 2009/12/09
49. Stanek G, Wormser GP, Gray J, Strle F (2012) Lyme borreliosis. *The Lancet* 379:461–473
50. Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JW, Li X, Mead PS (2016) Lyme borreliosis. *Nat Rev Dis Primers* Dec 15:2:16090. Epub 2016/12/16
51. Sun R, Peng M, Xu P, Huang F, Xie Y, Li J, Hong Y, Guo H, Liu Q, Zhu W (2020) Low-density lipoprotein receptor (LDLR) regulates NLRP3-mediated neuronal pyroptosis following cerebral ischemia/reperfusion injury. *J Neuroinflammation* Nov 5:17:330. Epub 2020/11/07
52. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P et al (2019) STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* Jan 8:47:D607–D613. Epub 2018/11/27
53. Takeuchi O, Kawai T, Sanjo H, Copeland NG, Gilbert DJ, Jenkins NA, Takeda K, Akira S (1999) TLR6: A novel member of an expanding toll-like receptor family. *Gene* Apr 29:231:59–65. Epub 1999/05/08
54. Thomas V, Fikrig E (2002) The Lyme disease vaccine takes its toll. *Vector Borne Zoonotic Dis* Winter 2:217–222. Epub 2003/06/14
55. Torre D, Lachmann A, Ma'ayan A (2018) BioJupies: Automated Generation of Interactive Notebooks for RNA-Seq Data Analysis in the Cloud. *Cell Syst* Nov 28;7:556–561 e553. Epub 2018/11/19
56. Wu J, Xiao J, Zhang Z, Wang X, Hu S, Yu J (2014) Ribogenomics: the science and knowledge of RNA. *Genomics Proteomics Bioinformatics* Apr 12:57–63. Epub 2014/04/29

57. Yao L, Gu X, Song Q, Wang X, Huang M, Hu M, Hou L, Kang T, Chen J, Chen H et al (2016) Nanoformulated alpha-mangostin ameliorates Alzheimer's disease neuropathology by elevating LDLR expression and accelerating amyloid-beta clearance. J Control Release Mar 28:226:1–14. Epub 2016/02/03

58. Zhang X, Yang L, Chien S, Lv Y (2018) Suspension state promotes metastasis of breast cancer cells by up-regulating cyclooxygenase-2. Theranostics 8:3722–3736. Epub 2018/08/08

59. Zhao H, Dai X, Han X, Liu A, Bao F, Bai R, Ji Z, Jian M, Ding Z, Abi ME et al (2019) Borrelia burgdorferi basic membrane protein A initiates proinflammatory chemokine storm in THP 1-derived macrophages via the receptors TLR1 and TLR2. Biomed Pharmacother Jul 115:108874. Epub 2019/04/20

60. Zhao Z, Tao L, Liu A, Ma M, Li H, Zhao H, Yang J, Wang S, Jin Y, Shao X et al (2018) NFkappaB is a key modulator in the signaling pathway of Borrelia burgdorferi BmpAinduced inflammatory chemokines in murine microglia BV2 cells. Mol Med Rep Apr 17:4953–4958. Epub 2018/02/03

Tables

Table 1 Primer Sequences Used in Quantitative Real-Time Polymerase Chain Reaction

Gene	Forward (5'®3')	Reverse (5'®3')	species
<i>TLR6</i>	TGAATGCAAAAACCCTTCACCT	CCAAGTCGTTTCTATGTGGTTGA	Homo sapiens
<i>ANGPT1</i>	TCGTGAGAGTACGACAGACCA	TCTCCGACTTCATGTTTTCCAC	Homo sapiens
<i>LDLR</i>	TGAACTGGTGTGAGAGGACC	TTTAGCCTGACGGTGGATGT	Homo sapiens
<i>SREBF1</i>	CTCAGATACCACCAGCGTCT	TTGCGATGCCTCCAGAAGTA	Homo sapiens
<i>TNC</i>	CTCCTCCCAAAGACCTCGTT	AAGTACTCCACACCAGGCTC	Homo sapiens
<i>ITGA</i>	TGGTCATCAGGGCACTATCC	CACTTGTCCAAAGGCACCAA	Homo sapiens
<i>β-Actin</i>	TGGCATCCACGAAACTACCT	CAATGCCAGGGTACATGGTG	Homo sapiens
<i>TLR6</i>	GACAAGGACACGGATTAGCCATC	CAGGTTGACACGGTGACAGTTCC	Macaca mulatta
<i>LDLR</i>	CTGGAAGAACTGGCGGCTGAAG	TGCGGCAAATGTGGACCTCATC	Macaca mulatta
<i>TNC</i>	GACAAGGACACGGATTAGCCATC	CAGGTTGACACGGTGACAGTTCC	Macaca mulatta
<i>GAPDH</i>	GCACCACCAACTGCTTAGCAC	TCTTCTGGGTGGCAGTGATG	Macaca mulatta

Table 2 The Hub Genes and their Description.

Gene symbol	Gene description	Differentially expression
<i>SELP</i>	Selectin P	Upregulated
<i>CD93</i>	CD93 Molecule	Upregulated
<i>ANGPT1</i>	Angiopoietin 1	Upregulated
<i>TLR6</i>	Toll Like Receptor 6	Upregulated
<i>SERPIND1</i>	Serpin Family D Member 1	Upregulated
<i>SREBF1</i>	Sterol Regulatory Element Binding Transcription Factor 1	Downregulated
<i>LDLR</i>	Low Density Lipoprotein Receptor	Downregulated
<i>MATN3</i>	Matrilin 3	Downregulated
<i>TNC</i>	Tenascin C	Downregulated
<i>ITGA2</i>	Integrin Subunit Alpha 2	Downregulated
<i>CSF1R</i>	Colony Stimulating Factor 1 Receptor	Downregulated

Table 3 Significantly enriched GO terms and KEGG pathways of the Hub DEG

Category	Term	Description	Count	P-Value	-ln(P-Value)
BP	GO:0030198	Extracellular matrix organization	3	0.004623509	2.335028301
BP	GO:0010867	Positive regulation of triglyceride biosynthetic process	2	0.005881637	2.230501758
BP	GO:0002687	Positive regulation of leukocyte migration	2	0.006414804	2.192816627
BP	GO:0031589	Cell-substrate adhesion	2	0.009076825	2.04206604
BP	GO:0071398	Cellular response to fatty acid	2	0.012262881	1.911407495
BP	GO:0006954	Inflammatory response	3	0.016468787	1.78333838
BP	GO:0007162	Negative regulation of cell adhesion	2	0.019661627	1.706380557
BP	GO:0045785	Positive regulation of cell adhesion	2	0.022817408	1.641733698
BP	GO:0007155	Cell adhesion	3	0.023632805	1.62648472
BP	GO:0030097	Hemopoiesis	2	0.031188723	1.506002404
BP	GO:0014068	Positive regulation of phosphatidylinositol 3-kinase signaling	2	0.034311493	1.464560382
BP	GO:0008203	Cholesterol metabolic process	2	0.03586952	1.445274431
BP	GO:0050729	Positive regulation of inflammatory response	2	0.038461267	1.414976408
BP	GO:0046718	Viral entry into host cell	2	0.042079309	1.3759314
CC	GO:0009986	Cell surface	4	0.001922057	2.716233687
CC	GO:0009897	External side of plasma membrane	3	0.004637261	2.333738458
CC	GO:0005886	Plasma membrane	7	0.005894117	2.229581248
CC	GO:0005887	Integral component of plasma membrane	4	0.027504704	1.560593032
MF	GO:0004872	Receptor activity	3	0.00557956	2.253400069
MF	GO:0001948	Glycoprotein binding	2	0.034133334	1.466821294
MF	GO:0001618	Virus receptor activity	2	0.036715556	1.435149885
KEGG	hsa04151	PI3K-Akt signaling pathway	4	0.003763234	2.424438754

GO, Gene Ontology. BP, biological process. CC, cellular component. MF, molecular function. KEGG,

Figures

Figure 1

Workflow of the study design. Abbreviations: DEGs, differentially expressed genes. PPI, protein-protein interaction. Bb, *Borrelia burgdorferi*. qRT-PCR, quantitative real-time polymerase chain reaction.

A

B

Figure 2

Volcano plot and Heatmap of differentially expressed genes identified in GSE85143. (A) The figure shows an interactive scatter plot displaying the $\log_2$ -fold changes and statistical significance of each gene calculated by performing a differential gene expression analysis. Every point in the plot represents a gene. Red points indicate significantly upregulated genes, blue points indicate downregulated genes. (B) The figure contains an interactive heatmap displaying gene expression for each sample in the GSE85143 dataset. Each row of the heatmap represents a gene, every column represents a sample, and every cell displays normalized gene expression values. The heatmap additionally features color bars beside each column which represent prior knowledge of each sample, such as the tissue of origin or experimental treatment.

Figure 3

Venn diagram of DEGs common to the Rhesus and GEO datasets. (A) Eighty shared upregulated genes in both the Rhesus and GEO dataset. (B) Thirty-two shared downregulated genes in both the Rhesus and GEO datasets. Abbreviations: DEG, differentially expressed gene. GEO, Gene Expression Omnibus.

Figure 4

GO annotation analysis of upregulated and downregulated DEGs. (A) GO annotation for upregulated DEGs. (B) GO annotation for downregulated DEGs. Abbreviations: GO, Gene Ontology. BP, biological process. CC, cellular component. MF, molecular function. DEG, differentially expressed gene. TFA, transcription factor activity.

Significant Enriched Pathway Terms

Figure 5

KEGG pathway enrichment analysis of upregulated DEGs. Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes. DEG, differentially expressed gene.

Figure 6

The PPI network of DEGs constructed using Cytoscape. Pink nodes indicate upregulated DEGs and green nodes indicate downregulated DEGs. Abbreviations: PPI, Protein-protein interaction. DEG, differentially expressed gene.

Bb group compared to Control group at 6 h, 12 h, and 48 h. (D) SREBF1 was demonstrated downregulated in Bb group compared to Control group at 6 h, 12 h, 24 h, and 48 h. (E) TNC was demonstrated downregulated in Bb group compared to Control group at 12 h and 48 h. (F) ITGA was demonstrated downregulated in Bb group compared to Control group at 48 h. Asterisks denote.*P-Value <0.05, **P-Value <0.01. Abbreviations: Bb, *Borrelia burgdorferi*. qPCR, quantitative real-time polymerase chain reaction.

Figure 9

The expression levels of indicated mRNA by qPCR comparing explants from the frontal cortex of rhesus brains co-cultured with live Bb with the controls at 6 h, 12 h, and 24 h. (A) TLR6 was demonstrated upregulated in Bb group compared to Control group at 24 h. (B-C) The expression levels of LDLR and TNC mRNA comparing explants from the frontal cortex of rhesus brains co-cultured with live Bb with the controls at 6 h, 12 h, and 24 h. Asterisks denote. **P-Value <0.01. Abbreviations: Bb, *Borrelia burgdorferi*. qPCR, quantitative real-time polymerase chain reaction.

Supplementary Files

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- [FigureS1.png](#)
- [FigureS2hsa04620.png](#)
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- [SupplementalFile1.xlsx](#)
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- [SupplementalFile3.txt](#)
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